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A METHOD OF CONTROLLING THE TEMPERATURE OF THE PARAFFIN BLOCK AND MICROTOME KNIFE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 188

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(WITH TWO FIGURES)

The successful production of a continuous ribbon of paraffin depends chiefly on the temperature of the block in which the object is imbedded, and of the microtome knife. The hardness and size of the object may of course affect the ribbon, but if the object is small, not refractory, and completely infiltrated, the effect is practically negligible. If the temperature of the knife and block is made sufficiently low, very thin sections can be cut without unduly compressing the object, regardless of the melting point of the paraffin.

With paraffin of a given melting point there is a definite temperature at which the block and knife must be kept in order to produce a continuous ribbon of definite thickness without injurious compression of the sections. If the microtome is set for thinner sections without lowering the temperature of the knife and block, the sections are compressed more and more as they are made thinner, until a point is reached where the tissues are crushed out of all resemblance to their original condition. This crushing of the sections has in some instances resulted in erroneous interpretation of structures even by otherwise competent investigators. Again, if the microtome is set for thicker sections and the temperature of the knife and block is not correspondingly raised, excellent sections result, but their edges refuse to weld and they come away singly, a source of much trouble when an absolutely unbroken series is required. If the thickness is further increased, the sections come away rolled so tightly that in some instances they cannot be unrolled even by floating on warm water.

In practice, when thick sections (10–20 μ) are wanted, the object is imbedded in paraffin melting at 45–52° C. If thinner sections are required (3–5 μ), paraffin melting at 58–62° C. is necessary. Even when the harder paraffin is used, in order to cut sections 2–4 μ thick, at ordinary room temperature, the knife and block must be cooled. If they are not cooled below the room temperature, which in winter is usually about 20–22° C., the sections are hopelessly crushed. In summer it becomes increasingly difficult to make thin sections.

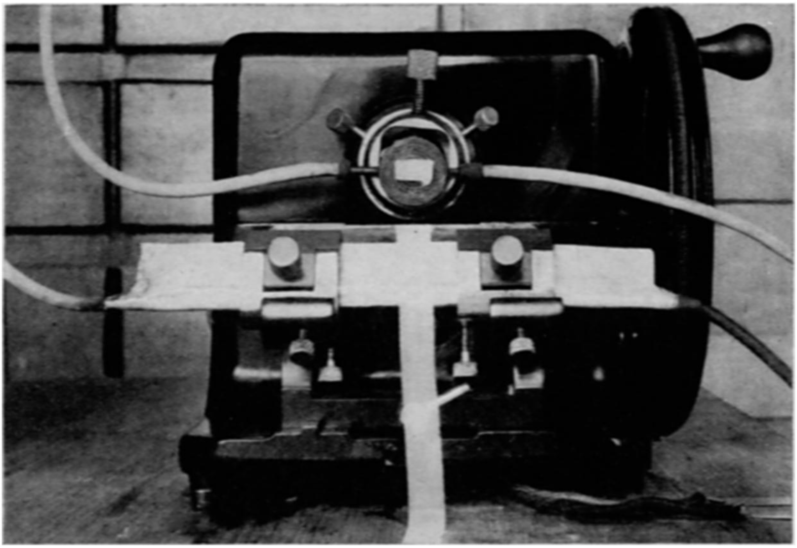

Fig. 1.—Apparatus for temperature control, showing object holder and cooling trough in place.

Many delicate plant tissues, notably certain liverworts, can be imbedded in paraffin melting at 45–52° C. with perceptibly little injury, but when the same material is imbedded at a temperature of 58–62° C. serious injury may result. For example, a tropical species of *Notothylas*, which at 52° showed little shrinkage, at 62° shrunk to nearly half the original thickness of the thallus. In addition to the action of heat, the effect of the large coefficient of expansion of paraffin (0.00027854) becomes marked when the harder paraffin is used. It follows that by using paraffin of a low melting point, the effect of heat and of the coefficient of expansion can be minimized.

In this laboratory the practice has been to cool the knife and block with lumps of ice. This method, while in the main giving good results, is unsatisfactory for the finest sectioning. Water usually gets on the ribbon and does damage; also great care is necessary to keep the microtome from rusting.

The various difficulties were in a large measure overcome as follows: The face of the ordinary circular metal object holder, which comes with all microtomes of the better class, was turned out to form a cup. A plate of thin brass was soldered over the cup, making a new face for the object holder, and turned down to the original diameter of the disk. Two holes were drilled and tapped at opposite sides of the disk and two short tubes for attaching small rubber tubes were screwed in. Fig. 1 shows this disk in the clamp of the microtome.

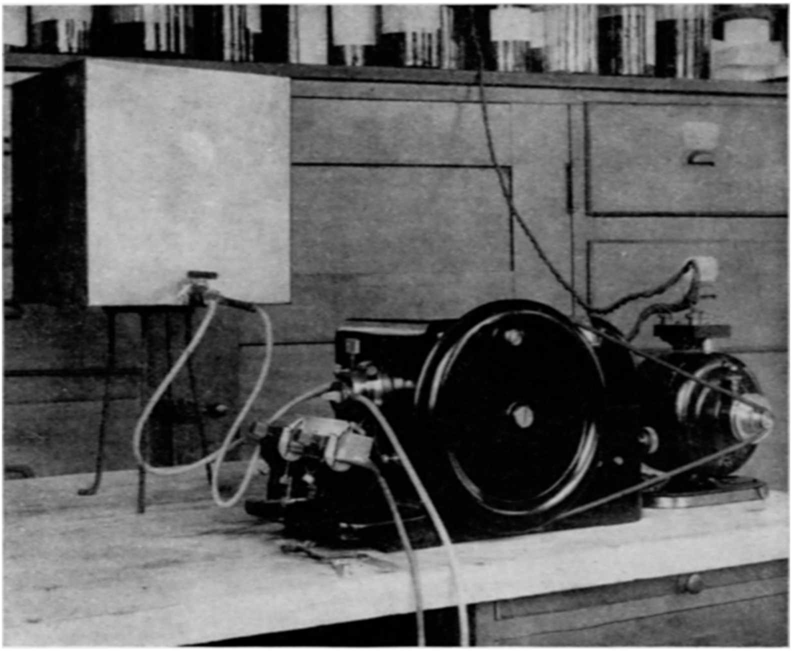

FIG. 2.—Apparatus for temperature control, showing all attachments

For controlling the temperature of the knife, a trough of thin metal—tin, brass, or copper—about 20 cm. long and about 2 cm.

deep was made to take in the knife and to permit about 1 cm. of the knife to project. A nipple at each end of the trough serves to attach small rubber tubes (fig. 1). The ends of the trough should be soldered to prevent leaking on the microtome. Any microtome knife having a detachable handle can be used. A flexible safety razor blade may be used, provided the heads of the clamping screws are flush with the sides of the blade holder.

A tank provided with a stopcock and Y-tube is placed above the microtome at a height which will insure a good flow of water. One tube of the "Y" is attached to a tube of the object holder by a rubber tube of small caliber, the other to the nipple of the cooling trough. Tubes lead from the holder and from the trough to a waste receptacle. The tank is filled with water of the proper temperature for the required thickness of ribbon. The block and knife reach the proper temperature for cutting soon after the water is turned on. The entire apparatus is shown in fig. 2.

The apparatus was designed primarily for cutting very thin sections (2–4 μ) of liverworts which had to be imbedded in soft paraffin, but it has been found useful when very thick sections (20–50 μ) are wanted. In cutting the latter, the knife trough is detached, and the tank filled with warm water which is allowed to flow through the object holder only. The temperature of water necessary for cutting sections of various thicknesses is easily determined by experiment.

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